--26. The method of claim 25, wherein the microorganism is *Escherichia coli*.--

--27. The method of claim 25, wherein the enzyme is glucose dehydrogenase.--

REMARKS

Claims 7-10, 12, 14, and 23-27 are now pending, claims 1-6, 11, 13, and 15-22 having been cancelled and new claims 23-27 added by the above amendments. Claims 1-6, 11, 13, and 15-18 have been cancelled as being drawn to an unelected invention. Claims 7-10 have been amended for clarity. The term "purified" introduced into claim 7 is supported at page 16, line 21, of the specification.[1] The term "hybridizes under stringent conditions" introduced into claim 10 is supported at pages 14-15, bridging sentence, of the specification. New claim 23 is supported at page 14, line 19, of the specification. New claim 24 is supported at pages 13-14, bridging paragraph, and at pages 31-34, Examples 12-16 of the specification. New claims 25-27 are fully supported by original claims 20-22 as filed, and by pages 31-34, Examples 12-16 of the specification. The term "isolated nucleic acid" in new claim 25 is supported at pages 14-15, bridging paragraph, of the specification. No new matter has been added by any of the above amendments.

The Invention

Applicant has discovered a new method for asymmetric reduction of a 4-halo-acetoacetic acid ester to produce an optically active (S)-4-halo-3-hydroxybutyric acid ester, using an acetoacetyl CoA reductase. The enzyme can be purified (e.g., as in claim 7) and/or recombinantly produced (e.g., as in claim 25). Until applicant's invention, it was not known that any acetoacetyl CoA reductase could be used to produce an optically active (S)-4-halo-3-hydroxybutyric acid ester.

[1]By "purified acetoacetyl-CoA reductase" applicant means the art-recognized process of removing other proteins that are naturally associated with the acetoacetyl CoA reductase in a cell. An enzyme can be purified from naturally occurring organisms or from organisms genetically engineered to produce the enzyme.

Rejections under 35 U.S.C. § 112, First Paragraph

I

Claims 7-10, 12, 14, and 19-22 are rejected because the specification allegedly fails to enable the full scope of the claims (pages 2-4, section 2 of the office action). Claim 19 has been cancelled, and claims 20-22 have been rewritten as new claims 25-27. Applicant traverses the rejection on the following grounds.

The Examiner is apparently concerned that the pending claims encompass the use of "any protein capable of reducing 4-halo-acetoacetic acid ester or its derivative to produce (S)-4-halo-3-hydroxybutyric acid ester" (e.g., at page 3, lines 7-8, of the office action). In addition, the Examiner contends that the specification enables the use of acetoacetyl CoA reductase only from *Ralstonia eutropha*, the amino acid sequence of which is given in SEQ ID NO:9 (page 3, lines 16-17, of the office action), but does not enable the use of enzymes in which a portion of their amino acid sequences have been modified or deleted (page 4, first full paragraph, of the office action).

It is first noted that the pending claims require the use of an acetoacetyl CoA reductase (i.e., a protein having this enzymatic activity), not any protein, as the Examiner states. Indeed, this requirement in each pending claim is fully commensurate with applicant's discovery that such enzymes can be used in the asymmetric reduction reaction.

Second, the Examiner's contention that the claims are enabled for the use of only SEQ ID NO:9 is without merit, given the substantial support in the specification for carrying out the claimed methods using other acetoacetyl CoA reductases. For example, a multitude of acetoacetyl CoA reductases are known to the skilled artisan and can be used in the claimed methods (see pages 13-14, bridging paragraph, of the specification). To confirm that other acetoacetyl CoA reductases also work as intended, applicant has cloned and characterized the acetoacetyl CoA reductase from *Zoogloea ramigera*, a source of enzyme recited at page 14, line 19, of the specification. The results of this study are described in the Declaration of Hiroaki Yamamoto under 37 C.F.R. § 1.132 ("Yamamoto Declaration"), which is submitted herewith. A signed copy of the declaration will be submitted as soon as possible.

As described at paragraph 11 and Table 3 of the Yamamoto Declaration, the *Zoogloea ramigera* enzyme asymmetrically reduced ethyl 4-chloroacetoacetate to (S)-ethyl 4-choloro-3-

hydroxybutyrate, with a yield and optical purity comparable or superior to what are described for the *Ralstonia eutropha* enzyme (page 34, Example 16 of the specification). Since the asymmetric reduction reactions using the *Zoogloea ramigera* enzyme were conducted using standard procedures known in the art of biochemistry and in accordance with the teachings of the specification (paragraphs 6-11 of the Yamamoto Declaration), applicant submits that the use of acetoacetyl CoA reductase from any biological source, including those recited in the specification, is fully enabled. Indeed, the Examiner has not provided any reason as to why the use of naturally occurring acetoacetyl CoA reductases and their fusion proteins (e.g., to facilitate purification after recombinant production) would not be enabled. New claim 24 has been added to specifically cover the latter aspect of the invention.

Third, in regard to the Examiner's concern that the specification does not enable the use of an acetoacetyl CoA reductase in which the amino acid sequence has been altered, applicant notes that mutagenesis techniques are well known in the art, which apparently the Examiner admits (page 3, line 18, of the office action). Nevertheless, the Examiner states that "it is not routine in the art to screen for multiple substitutions . . . or multiple modifications" (pages 3-4, bridging sentence). It is noted that the federal courts have stated in regard to enablement that "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988), citing *In re Jackson*, 217 USPQ 804, 807 (Bd. Pat. App. & Int. 1986). Here, not only are the methods for producing mutant enzymes routine to the skilled artisan, but the specification further provides specific methods that can be used to generate the mutants (page 15, lines 8-15, of the specification). Having produced mutant enzymes, the skilled artisan is further armed by the specification with the functional assays (e.g., Examples 12-16 of the specification) necessary to screen each mutant for its ability to asymmetrically reduce a 4-halo-acetoacetic acid ester into a (S)-4-halo-3-hydroxybutyric acid ester. Consequently, applicant has met the legal standard for enablement of the pending claims.

For the reasons discussed above, the enablement rejection should be withdrawn.

II

Under the written description requirement, claims 7-10 and 19-22 are rejected for what the Examiner characterizes as a failure to describe in the specification a representative number of protein species useful in the invention (page 5, section 3 of the office action). The Examiner contends that the specification provides only one representative species of the genus of all proteins useful in the claimed asymmetric reduction reactions. Applicant traverses on the ground that, contrary to the Examiner's contention, numerous species are described in the specification and, in any event, are known to the skilled artisan.

As discussed in the previous section, the Examiner's concern that the specification fails to describe all possible proteins capable of asymmetric reduction of a 4-halo-acetoacetic acid ester is misplaced, because the pending claims are limited to the use of an acetoacetyl CoA reductase, not "any protein." Thus, it is within the genus of acetoacetyl CoA reductases that the analysis of representative species should begin.

The specification provides more than 20 examples of acetoacetyl CoA reductases from a diverse array of organisms that can be used in the methods of the invention (pages 13-14, bridging paragraph, of the specification). The results described in the Yamamoto Declaration confirm that other acetoacetyl CoA reductases work in the asymmetric reduction reactions. Consequently, the Examiner's contention that only one representative species is disclosed in the specification is erroneous.

In regard to the alleged failure of the specification to disclose a structure/function relationship for the genus of enzymes recited in the claims, applicant notes that the acetoacetyl CoA reductase activity is associated with each of the more than 20 examples in the specification. Further, a representative number of these examples have been cloned and sequence, and are therefore available to the skilled artisan for the practice of the invention.[2] Thus, the "relationship" between structure and function is inherent in the diversity and conservation of amino acid sequences of these representative enzymes. The skilled artisan need merely to view these amino acid sequences in alignment to determine which regions of the consensus amino acid

[2]The acetoacetyl CoA reductase source and the GenBank Accession No. in parenthesis are as follows: *Acinetobacter sp.* RA3849 (L37761), *Alcaligenes latus* (AF078795), *Alcaligenes sp.* SH-69 (AF002014), *Azospirillum brasilense* (X64772), *Chromatium vinosum* strain D (1172473), *Paracoccus denitrificans* (D49362), *Pseudomonas sp.* (Z80156), and *Sinorhizobium meliloti* 41/*Rhizobium meliloti* (U17226).

sequence are important for activity. The raw sequences and tools, including software, for these alignments are available to the skilled artisan (see, e.g., the tools available at http://www.ncbi.nlm.nih.gov/, or its past embodiments).

Thus, the specification fully conveys to one skilled in the art that the inventor had possession of the claimed invention by providing sufficient representative examples (more than 20) of acetoacetyl CoA reductases suitable for use in the claimed methods. Consequently, the rejection should be withdrawn.

Rejection under 35 U.S.C. § 112, Second Paragraph

Claim 10 has been rejected for reciting the allegedly indefinite term "hybridizable." Applicant has replaced this term with "hybridizes under stringent conditions," a term of art that particularly defines a set of hybridization conditions known to the skilled artisan. Consequently, the rejection should be withdrawn.

Rejections under 35 U.S.C. §§ 102 and 103(a)

I

Claims 7, 10, 12, and 19 are rejected under 35 U.S.C. § 102(b) as anticipated by, or alternatively under 35 U.S.C. § 103(a) as obvious over, Matsuyama et al. (U.S. Patent No. 5,559,030) at pages 7, section 7 of the office action. Claim 19 has been cancelled. Claims 7 and 25 are the only pending independent claims. Applicant has overcome this rejection by amendment to claim 7.

Matsuyama describes methods of asymmetrically reducing 4-halo-acetoacetic acid esters to (S)-4-halo-2-hydroxybuyric acid esters (e.g., col. 1, lines 53-63), using a non-recombinant bacterium selected from a list of genera and species (col. 3, line 55, to col. 4, line 59). The reference does not describe using a purified acetoacetyl CoA reductase from a biological source for the same purpose. At best, Matsuyama describes disrupted suspensions of non-recombinant cells (col. 6, lines 11-14), but fails to describe any preparation in which acetoacetyl CoA has been purified from the proteins with which it is naturally associated.

Amended claim 7 now requires asymmetric reduction using a purified acetoacetyl CoA reductase. Since Matsuyama does not describe the use of a purified acetoacetyl CoA reductase nor provide any motivation for or guidance to enable such use, claim 7 is patentable over

Matsuyama. Indeed, it is applicant and not Matsuyama that recognized the utility of acetoacetyl CoA reductases in asymmetric reduction of 4-halo-acetoacetic acid esters.

New claim 25 requires the use of a microorganism transformed with an isolated nucleic acid encoding an acetoacetyl CoA reductase, or a cell-free fraction thereof, and expression of the recombinant acetoacetyl CoA in the microorganism. Since Matsuyama does not describe the use of such a transformed microorganism or cell-free fraction thereof, nor provide any motivation for such use, claim 25 is also patentable over Matsuyama.

As claims 7 and 25 are patentable over Matsuyama, so are claims 8-10, 12, 14, 23, 24, 26, and 27, all of which directly or indirectly depend from claim 7 or 25.

II

Claims 7 and 10 are rejected under 35 U.S.C. § 102(e) as being anticipated by Kimoto et al. (U.S. Patent No. 6,001,618; filed December 22, 1998). Applicant submits herewith a certified translation of Japanese Patent Application No. Hei 10-300178, filed October 21, 1998, from which the present application claims priority (see Combined Declaration and Power of Attorney filed September 20, 1999). This translation verifies that the pending claims are fully supported by the Japanese priority document and are therefore entitled to the October 21, 1998 priority date. Having established a priority date of October 21, 1998 or earlier for the present application, applicant has removed Kimoto as 102(e) prior art. Therefore, the rejection should be withdrawn.

III

Claims 8, 9, 14, and 20-22 are rejected as obvious over Matsuyama in view of Peoples et al. (U.S. Patent No. 5,229,279) or Summerville et al. (WO 93/02187). Matsuyama is described above. Peoples and Summerville are cited to show that the acetoacetyl CoA reductase enzyme from *Ralstonia eutropha* had been cloned and sequenced. Claims 8, 9, and 14 depend from claim 7. Applicant traverses this rejection on the ground that there is no motivation to combine the cited references in any manner sufficient to produce the claimed methods.

As discussed above, Matsuyama fails to recognize the new, unexpected asymmetric reduction activity associated with a purified acetoacetyl CoA reductase (recited in claim 7), a microorganism that recombinantly expresses an acetoacetyl CoA reductase (recited in claim 25), or a cell-free fraction thereof (recited in claim 25). Neither Peoples nor Summerville alleviate

this deficiency in Matsuyama. Instead, both Peoples and Summerville merely recognize the role of acetoacetyl CoA reductase in the multi-step production of polyhydroxybutyrate (Fig. 1 of Peoples; page 8, line 29, to page 10, line 8, of Summerville). Thus, there is no motivation whatsoever to combine an acetoacetyl CoA reductase (such as described in Peoples or Summerville) with a method of asymmetrically reducing a 4-halo-3-hydroxybutyric acid ester (as described in Matsuyama).

Nevertheless, the Examiner states that the motivation for combining these references is that "these nucleic acids encoding these enzymes have been cloned and therefore this allows for more control in the reaction conditions and the opportunity to improve the enzyme by the use of recombinant technologies and mutagenesis" (page 10, lines 1-4, of the office action). Such a general motivation that would apply to any use of any previously cloned protein is hardly sufficient to render obvious a method claim directed to a particular narrow use (here, asymmetric reduction of a 4-halo-acetoacetic acid ester). The federal courts have long held that a claim is not obvious over a combination of references unless there is specific motivation to combine the portions of the references necessary to arrive at the claimed invention. See, e.g., *In re Rouffet*, 47 USPQ2d 1453 (Fed. Cir. 1998) and authority cited therein. In the present case, no such specific motivation exists. Instead, the Examiner appears to have used applicant's own disclosure to pick and choose unrelated descriptions in the prior art to arrive at the claimed invention. Time and again, the federal courts have confirmed that this "hindsight reconstruction" cannot be used to establish obviousness. *Id.*

Because of the lack of motivation to combine Matsuyama with Peoples or Summerville, claims 7 and 25 are nonobvious over the cited references. As claims 7 and 25 are nonobvious over the cited references, so are all claims dependent thereon.

CONCLUSION

Applicant submits that all of the claims are now in condition for allowance, which action is requested. Filed herewith is a Petition for Automatic Extension with the required fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 8-10-00

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